NZ01/06

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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 15 August 2000 with an application for Letters Patent number 506337 made by DIATRANZ LIMITED.

Dated 1 February 2001.

Neville Harris
Commissioner of Patents

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506337

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

"Xenotransplantation Preparations and Their Use"

We, DIATRANZ LIMITED, a company duly incorporated under the laws of New Zealand of 19 Laureston Avenue, Papatoetoe, Auckland, New Zealand, do hereby declare this invention to be described in the following statement:

The present invention relates to improvements in and/or relating to the treatment of diabetes using xenotransplantation and more particularly preparations and their use.

The present invention relates to the treatment of a mammalian patient suffering from diabetes (including humans) which involves the transplantation into the mammal of viable confined or encapsulated porcine islets capable of producing insulin within its host.

Type 1 (insulin-dependent) diabetes mellitus is a common endocrine disorder that results in substantial morbidity and mortality, and leads to considerable financial costs to individual patients and healthcare systems.

Treatment with insulin, while life-saving, often does not provide sufficient control of blood glucose to prevent the feared complications of the disease, which has provided the impetus for intensive research into better methods of sustaining normoglycaemia.

Among the newer treatment strategies that have been proposed, transplantation of pancreatic β islet cells, obtained either from other humans or animals, has received the most attention worldwide. This is because transplantation can restore not only the insulin-secreting unit, but also the precise fine tuning of insulin release in response to multiple neural and humoral signals arising within and beyond the islets of Langerhans.

Human islet cell transplantation is limited by the shortage of human islet tissue. The use of pig islet cells is currently viewed as the most promising alternative since:

(a) the supply of pig cells can be readily expanded by optimising the supply of donor animals;

(b) pig and human insulin have close structural similarities; and

(c) physiological glucose levels in pigs are similar to those in humans.

The rationale for this treatment approach (termed 'xenotransplantation') is that the implanted pig islets have the potential to mimic the normal physiological insulin response in type 1 diabetics such that near-normal blood glucose levels may be achievable without insulin administration or with a reduced requirement for it. As a consequence, long-term diabetes complications may be prevented and patients should experience less hypoglycaemia than they do with the currently recommended 'intensive' insulin regimens.

The present invention recognises the ability to source appropriate islets from piglets at or near full term gestation and to convert those into an appropriate xenotransplantable source of islets with viability in a human being by following certain procedures in respect of the harvesting and extraction of the islets, the treatment of the islets prior to xenotransplantation as well as regimes of use of such islets.

In one aspect the invention consists in **a method of preparing porcine islets to provide viable xenotransplantable islets for a human** which comprises the steps of

(i) harvesting the pancreas of piglets at or near full term gestation (ie; at from -20 to +10 days full term gestation and most preferably from -7 to +10 days

full gestation),

(ii) extracting the islets from such harvested pancreas using Liberase™ (either porcine Liberase™ or Human Liberase™).

Preferably said pancreas and/or said islets are subject to a trauma protecting agent selected from suitable anaesthetic agents such as, for example, lignocaine.

Preferably said pancreas are subjected to a mechanical disruption prior to or during exposure to said Liberase™.

Preferably human Liberase™ (Liberase HI™) is utilised in the step (ii) digestion process.

We have determined that much greater yields per neonatal pig pancreas can be obtained using either pig or human Liberase™ (eg; sourced in New Zealand from Roche) rather than collagenase.

Whilst there is disclosure in "*Improved Pig Islet Yield and Post-Culture Recovery Using Liberase P1 Purified Enzyme Blend*", T J Cavanagh et al. Transplantation Proceedings 30, 367 (1998) and in "*Significant Progress In Porcine Islets Mass Isolation Utilizing Liberase HI For Enzymatic Low-Temperature Pancreas Digestion*", H. Brandhorst et al. Transplantation Vol 68, 355-361 No. 3, August 15, 1999 the yields therefore therein are low compared to those we have discovered. If, for example, in following the procedure of Brandhorst et al. there is a yield increase of islets over collagenase of from 400 to say 800 with the procedure using human Liberase™ (ie; Liberase™ HI) as in the Brandhorst et al. procedure but confined to neonatal porcine islets such as those as 7 days post delivery extra ordinarily larger yields are possible, namely, the equivalent to from 400 which would be the case with crude collagenase to 30000 which as can be seen as very much greater than that to be expected from following the procedure of Brandhorst et al. with pigs.

Preferably said procedure involves subsequently or during the aforementioned method treating the islets with the N-Terminal Tripeptide of IgF_1 (eg; glycine-proline-glutamate usually preferred to as "GPE").

Preferably said islets are treated (preferably after extraction from the pancreas) with nicotinamide.

In another aspect the invention is any one of the following

(i) **an islet isolation and preparation procedure,**

(ii) **an encapsulated or confined islet providing procedure;**

(iii) **a method** resulting in (i) or (ii) and (iv) **related uses or methods of use,** each of (i), (ii), (iii) and (iv) being substantially as in Figure 1 hereof.

In another aspect therefore the present invention consists in **a method of enhancing the yield of viable porcine islets from piglets using Liberase™** which involves

confining the extraction procedure to piglets of from -20 to +10 full days gestation or to piglets close to their usual full term gestation.

In a further aspect the present invention consists in **any isolated porcine islets or xenotransplantable preparations including viable porcine islets** where the digestion has been in accordance with the method in accordance with the present invention.

Preferably said islets have been mechanically treated only following application of a suitable anaesthetic to the pancreatic tissue.

In another aspect the present invention consists in **xenotransplantable preparations of viable insulin producing islets** where the islets treated with nicotinamide and either IgF_1 or GPE have been extracted from the pancreas of piglets at or near full term gestation, eg; preferably from -20 to +10 days full term gestation.

Preferably said preparation includes a suitable antibiotic.

Preferably said suitable antibiotic is ciproxin although if desired strepomycin may be used. It has been found however that ciproxin is both a better antibiotic and toxic to the islets than strepomycin.

In another aspect the invention consists in **a method for treatment of a mammalian patient** suffering from diabetes which comprises:

(a) extracting pancreatic β islet cells from piglets at or near full term gestation;

(b) treating said islets with nicotinamide,

(c) encapsulating or otherwise confining said islets in a biocompatible material which will allow *in vivo* glucose movement to and insulin movement from the islets, and

(d) injecting or otherwise implanting the encapsulated or confined islet cells of step (c) so as to transplant into said mammalian patient an effective amount of viable piglet islet cells capable of producing insulin in the patient, and

(e) (optionally) administering nicotinamide to said mammalian patient at least subsequent to transplantation; and

(f) (optionally) administering a casein-free (as herein defined) diet to said mammalian patient.

Preferably step (a) involves the use of porcine or human Liberase™ (preferably human Liberase™) and preferably the preliminary exposure of the pancreatic tissue at or prior to such digestion with Liberase™ to a suitable anaesthetic, such as lignocaine.

Preferably step (b) follows, includes or is followed by a procedure of treating said islets with IgF_1 and/or the GPE.

Preferably said piglets at or near full term gestation from which the pancreatic β islet cells are extracted are at from -20 to +10 days full term gestation.

Preferably said piglets are at from -7 to +10 days full term gestation.

The Encapsulation Option:

In one form said islets may be encapsulated.

Preferably said encapsulation is with an alginate material (preferably sodium alginate) (whether after pre-coating or not with some other material).

Preferably said alginate (preferably sodium alginate) is in ultra pure form.

Preferably each islet or grouping of islets is entrapped in an *in vivo* insulin and glucose porous biocompatible alginate or alginate like surround.

Preferably such coating prevents, once implanted, direct tissue contact with said islets and/or any pre-coating matrix which itself has the requisite porosity once implanted.

Preferably each encapsulation involves presenting islets and a suitable alginate solution into a source of compatible cations thereby to entrap the islets in a cation - alginate gel.

Preferably said cation alginate gel is calcium-alginate gel.

Preferably said alginate used in the solution is sodium alginate.

Preferably the islets and sodium alginate solution (preferably 1.6% w/w) is presented as a droplet (eg. through a droplet generating needle) into a bath of suitable cations (eg. gelating cations such as calcium chloride).

Preferably the gel encased islets are coated with a positively charged material and thereafter optionally are provided with an outer coat of a suitable alginate.

Preferably said positive charging material is poly-L-ornithine.

Preferably the gel entrapping the islets within the outer coating is then liquified.

Preferably said liquification is by the addition of sodium citrate.

Preferably said capsules contain a plurality of islet cells (preferably about three) and preferably have a diameter of from about 300 to 400 microns.

After liquification of the alignate entrapping the islets, the "capsules" are washed, and again coated with alignate which neutralizes any residual change on the poly-L-ornithine coating and prevents direct contact of the poly-L-ornithine with tissues when the entire capsule is transplanted.

Preferably the alginate production process has involved the following steps:

Seaweed harvest→Washing→Alginate extraction→Filtration (preferably a 0.2 μm filter→Precipitation→Drying.

Preferably the ultrapure alginate is Kelco LV produced by Monsanto-Kelco, US and has the following specifications;

1. Viscosity: 2% - 100-300 cps (Brookfield 25°C, speed 3,60 rpm)
2. pH: 6.4-8.0
3. Protein content <0.5%

4. Filtration: through 0.2 μm
5. Chemical analysis:

Ca: <100 ppm	Mg <40 ppm	Mn: <10 ppm
Cu: <40 ppm	Zn: <40 ppm	Sr: <40 ppm
Fe: <60 ppm	Pb: <50 ppm	As: <100ppb
Hg: <40 ppb	Si: <10 ppm	

6. Endotoxin level - measured by LAL test (at University of Perugia): 39 EU/g [NB. Any level below 100 EU/g in this test is considered endotoxin-free].
7. Molecular weight: 120,000 - 190,000 kD
8. Mannuronic acid (M) content: M fraction (F_m) 61%
9. Guluronic acid (G) content: G fraction (F_G) 39%

Preferably the filtration has been with a multiple filtration process employing positively charged filters that remove any lipopolysaccharide content.

The Vascularised Subcutaneous Collagen Tube Confinement Option:

In another form said islets may be confined in a suitable delivery device or vehicle, eg; a suitable vascularised subcutaneous collagen tube using Sertoli cells as the method of preventing tissue immune rejection.

Preferably said pancreatic β islet cells at some stage after extraction from the piglets and prior to encapsulation are exposed to IgF_1 or at least the N-Terminal Tripeptide (eg; glycine-proline-glutamate hereafter referred to as "GPE").

Preferably said exposure to IgF_1 or GPE is greater for those cells from piglets furthest from full term gestation but preferably there is exposure to IgF_1 or GPE for all cells extracted irrespective of their relationship to full term gestation.

Preferably said extraction involves the use of a trauma protecting agent for the islet cells during the isolation and/or preparation thereof for encapsulation or confinement thereof, eg; in a collagen tube.

Preferably said agent is a trauma protecting agent selected from suitable anaesthetic agents such as, for example, lignocaine.

Preferably the mammalian patient is administered nicotinamide prior to transplantation and preferably after transplantation.

Preferably the casein-free as herein defined diet is administered to the mammalian patient at least after transplantation.

Preferably a casein-free as herein defined diet is administered prior to transplantation.

In a further aspect the present invention consists in **encapsulated pancreatic islets** of a kind useful in a method aforesaid.

In still a further aspect the present invention consists in **a method of porcine β islet cell production and/or method of xenotransplation thereof in an encapsulated or confined form** when preformed by a procedure substantially as hereinbefore described and/or substantially as hereinafter described and/or as shown in Figure 1 of the accompanying drawings.

In still a further aspect the present invention consist in a **xenotransplantable capsule** of at least one porcine pancreatic β islet cell comprising at least one viable porcine pancreatic β islet cell enclosed in an *in vivo* glucose porous and insulin porous biocompatible material.

As used herein "administering" includes self administering.

"Casein-free" as used herein refers to milk which does not contain a diabetogenic factor, particularly to milk containing no variant of β-casein which stimulates diabetogenic activity in humans. With reference to International PCT Application WO 96/14577, a non-diabetogenic variant for example, may be the A2 variant of β-casein. The full contents of PCT/NZ95/00114 (WO 96/14577) and PCT/NZ96/00039 (WO 96/36239) are here included by way of reference.

Preferred forms of the present invention will now be described with reference to the accompanying drawings in which

Figure 1 shows a preferred procedure for harvesting, isolating and preparing islet cells (with either confinement or encapsulation) and the associated treatment regime for a diabetic human patient in order to receive ongoing benefit from the xenotransplantation,

Figure 2 shows the effect of collagenase from various sources on islet yield and function,

Figure 3 shows the stimulation index of Liberase™ against Collagenase clearly showing that Liberase™ preparations (both human and porcine at suitable concentrations) gave higher yields and function in vitro than an optimised concentration of Collagense P,

Figure 4 shows the stimulation index of free islets when comparing the use of ciproxin against a penicillin/streptomycin mix and against a control of no antibiotics,

Figure 5 shows a plot of confined piglet islets (eg; using a vascularised subcutaneous tube collagen tube) and the post transplant relationship of blood glucose against insulin requirement in a 15 year old human insulin dependent diabetic patient, and

Figure 6 shows a plot of porcine c-peptide in response to IV glucose in the subject of Figure 5.

The major advantage of porcine islet cell transplantation over human islet cell transplantation is that the islet cell source can be readily expanded, and the biosafety of the cells can be thoroughly explored prior to transplantation. From a practical viewpoint, pancreas removal and islet cell isolation can be performed expeditiously in an ideal environment.

Important considerations relevant to the use of porcine islet cells in transplantation approaches for type 1 diabetes include the following:

- The structural and biological similarities of porcine and human insulin
- The fact that porcine insulin has been used to treat diabetes for several decades (and has only been replaced by human sequence insulin relatively recently); and
- The similarity of physiological glucose levels in pigs and humans. (Weir & Bonner-Weir 1997). This effectively means that pig islet cells can be expected to react similarly to their human counterparts in maintaining equivalent blood glucose concentrations.

1. ***The nature of the disease causing diabetes:*** successful long-term allotransplantation of human islets can be achieved in over 80% of patients when the disease is caused by non-immune processes. In contrast, even islets obtained from a non-diabetic twin cannot reverse autoimmune diabetes long-term in the diabetic twin member. This emphasises the critical role of autoimmunity in the failure of islet transplantation. This observation has been validated in allotransplantation of rodents with diabetes caused by autoimmunity as compared with diabetes due to pancreatectomy or chemical β cell destruction. No large animal model of autoimmune diabetes exists.

It is possible that the use of islets from different species (xenotransplantation) could avoid autoimmune destruction of transplanted islets, as the immune process of xenotransplant rejection is different to that of allotransplant rejection, but this is entirely hypothetical in humans.

2. ***Isolation and Preparation of Porcine Islet Cells for Xenotransplantation***

- **Animal Source and Transportation**

All animals intended as a source of pancreatic tissue for xenotransplantation are obtained from a specific pathogen-free (SPF) pig breeding facility which is maintained in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC). The facility maintains a high-health status colony with excellent standards of husbandry, and operates a record system that is readily accessible and archived indefinitely. Donor sows and sires are selected with the underlying objective of producing strong heterosis in donor litters.

- **Isolation and Purification of Islet Cells**

Following surgical removal, the donor pancreases are transferred to a clean room facility for further processing in a cold plastic container in 50ml tubes containing cold Hanks' Balanced Salt Solution (HBSS) with 0.2% human serum albumin (HSA) added. Blood samples from each donor are sent for virology testing

and toxoplasma serology. Samples from each organ are kept in a freezer at –80°C for future testing if necessary.

- ***Digestion***

The islet cells are isolated by standard collagenase digestion of the minced pancreas via the procedure documented by Ricordi et al. (1990), though with some modifications. Using aseptic technique, the glands are distended with Liberase™ (1.5 mg/ml), trimmed of excess fat, blood vessels and connective tissue, minced, and digested at 37°C in a shaking water bath for 15 minutes at 120 rpm. The digestion is done using Lignocaine mixed with the Liberase™ solution to avoid cell damage during digestion.

Following the digestion process, the cells are passed through a sterile 400μm mesh into a sterile beaker. A second digestion process is used for any undigested tissue.

- ***Washing and Culture***

The digested tissue is washed three times, and seeded into cell culture media RPMI 1640 to which is added 2% human serum albumin (HSA), 10 mmol/L nicotinamide, and antibiotic (Ciproxin).

- ***Quality Control Procedures***

To exclude any contamination of the tissue, quality control procedures are undertaken on cell culture samples after isolation and before encapsulation (further details are given in SOP P101). Three days after isolation, the cell culture is tested for microbiological contamination by accredited laboratories. Testing for porcine endogenous retrovirus (PERV) is undertaken at the Virology Laboratory, Auckland Hospital.

The *islet yield* is determined via dithizone (DTZ) staining of the cells, as specified in SOP Q200. Dithizone is a zinc-chelating agent and a supravital stain that selectively stains zinc in the islets of Langherhans, producing a distinctive red appearance.

The *viability* of the islet cells is determined using acridin orange and propidium iodide, as specified in SOP Q201. Acridin orange is a fluorescent stain that readily passes through all cell membranes to stain the cytoplasm and nucleus. Bright green fluorescence in both the nucleus and cytoplasm on exposure to ultraviolet (UV) light denotes intact live cells. Conversely, propidium iodide is a fluorescent stain that cannot pass through an intact membrane. It emits a bright red fluorescence when exposed to UV light, and the presence of propidium iodide in a cell nucleus indicates severe

damage or a dead cell.

- **Determination of *in vitro* Insulin Secretory Capacity**

Static glucose stimulation (SGS) is used to assess *in vitro* function of the porcine islets by exposing them to low and high concentrations of glucose and theophylline. Determination of the *in vitro* insulin secretory capacity is undertaken on both free islets (after 3 days in culture) and after their subsequent encapsulation or confinement.

- **Alginate Encapsulation Procedure**

Sodium alginate used for this procedure is extracted from raw material sources (seaweed) and prepared in a powdered ultrapure form. The sterile sodium alginate solution (1.6%) is then utilised at the Diatranz Islet Transplant Centre to manufacture encapsulated islets.

The encapsulation procedure (University of Perugia) involves extruding a mixture of islets and sodium alginate solution (1.6%) through a droplet generating needle into a bath of gelling cations (calcium chloride). The islets entrapped in the calcium-alginate gel are then coated with positively charged poly-L-ornithine followed by an outer coat of alginate (0.05%). The central core of alginate is then liquefied by the addition of sodium citrate. Most capsules contain 3 islet cells and have a diameter of 300 to 400μm.

The encapsulated islets are kept in cell culture, and then checked for contamination, insulin release and viability before transplantation. They are only released for transplantation if all quality control tests are negative.

- **Vasularised Subcutaneous collagen tube procedure:**

The islets may be implanted in a suitably vascularised subcutaneous collagen tube using Sertoli cells as the method of preventing tissue immune rejection.

In brief a closed ended tube of stainless steel mesh containing a loosely fitting Teflon rod is inserted subcutaneously in the intended graft recipient. Six weeks later the rod is removed—leaving a highly vascularised tube of collagen. A mixture the islets prepared as above together with Sertoli cells prepared by the method of Rajotte from the testes of the same piglets from whom the islets were obtained. The Sertoli cells are mixed with the islets in a ratio of about 1,000 cells /islet and inserted into the vascular tube. Which is then sealed with a Teflon stopper.

The effect of such a device containing piglet islets (approx 300,000) +Sertoli cells on insulin requirement in a 15 year old human insulin dependent diabetic subject is shown in Figure 5.

The secretion of porcine C-peptide in response to IV glucose in this subject 4

weeks after transplantation is shown in the Figure 6 below.

3. ***The viability of the islets:***

The processes by which islets are purified prior to transplantation are traumatic to these highly specialised tissues. Such trauma can induce necrosis or apoptosis – the latter being quite delayed.

Further trauma may result from encapsulation. Processes used by us in both the preparation of islets and their encapsulation have been optimised to ensure minimal damage to the islets. Such procedures have ensured zero warm ischaemia (compared with hours with most human islet preparations), have involved the use of nicotinamide to enhance successful *in vitro* explantation, have involved minimal incubation time with collagenase or Liberase™, have involved swift non-traumatic encapsulation technology, have involved (or involves) the use of IgF_1 (or the GPE tripeptide thereof), the use of an anaesthetic such as lignocaine, and the use of an antibiotic such as ciproxin. etc.

Our preferred preparation preferably uses neonatal (7-day old) islets which is crucial in both limiting islet trauma during purification, and assuring sufficient maturation of the islets for stimulated insulin production to occur after transplantation, eg; by injection or subcutanaceous placement.

4. ***Drugs used in the recipient:*** transplantation does not require and avoids the need for cytotoxic agents to suppress the immune system. Such agents are able to enter the alginate microcapsule and cause islet toxicity, as well as causing systemic toxicity. Instead, nicotinamide and the special diet disclosed is used.

Four type 1 diabetic adolescents received 10,000 free islets/kg bodyweight by intraperitoneal injection. The islets were located from term piglets using the standard collagenase digestion, purification and culture techniques described in section 3.2. All four recipients received oral nicotinamide (1.5 g/day) and a casein-free as herein defined diet both pre- and post-transplantation. A prompt reduction in insulin requirements, which was not clearly dose-related, was noted in the first week after transplantation. The reduction in insulin dosage range from 21 to 32%, and the response lasted for up to 14 weeks without encapsulation or confinement. However, insulin doses subsequently returned to their previous levels with such non protected islets.

The most likely reason for the transplant failure in these patients was chronic rejection. However, no adverse effects were noted.

We have now shown alginate-encapsulated porcine islet cell transplants in two human diabetic patients, prolonged functioning of the transplants. The islets were

transplanted by intraperitoneal injection, one patient receiving 15,000 IEQ/kg (total 1,300,000 islets) and the other 10,000 IEQ/kg (total 930,000 islets). Both patients were treated pre- and post-transplantation with oral nicotinamide and a soy-based/casein-free as herein defined diet. The preferred procedure as shown in Figure 1 was used for the preparation, the encapsulation being as aforesaid. Islet cells of -7 days to +10 days full gestation were used.

The preferred procedure as shown in Figure 1 was used for the preparation, the encapsulation being as aforesaid. Islet cells of -7 days to +10 days full gestation were used.

We have also shown ongoing efficacy with the collagen tube type confinement of the islets in humans.

- **IgF_1 or GPE Role:**

Porcine islets in culture which were exposed to IGF incorporating the amino terminal glycine-proline-flutamate (hereafter IGF-$_1$) (GPE)), increased their insulin response to glucose, by up to a 3-fold increase.

Table 1

	Incubated 24hrs with 0.1ug/ml IGF-1(GPE) after isolation	**CONTROL no IGF-1(GPE)**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 3 days culture Post isolation	236uU/hr/100IEQ	75.2uU/hr/100IEQ

- A concentration of 0.1ug/ml IGF-1(GPE) in culture is sufficient to produce optimal insulin secretion during glucose challenge. No further benefit was achieved by increasing the concentration of IGF-1(GPE).

	Incubated 24hrs with 0.1ug/ml IGF-1 (GPE)	**Incubated 24hrs with 1.0 ug/ml IGF-1(GPE)**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 3 days culture Post isolation	58uU/hr/100IEQ	56.8uU/hr/100IEQ

- Variations on the duration of IGF-1(GPE) exposure were tried on the porcine islet cells. However no increased benefit was found on culturing the islets with IGF-1(GPE) beyond a 24hrs period, post isolation.

	Incubated 7 days With 0.1ug/ml IGF-1(GPE)	**Incubated 24 hrs with 0.1ug/ml IGF-1(GPE)**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline 7days post isolation	58uU/hr/100IEQ	57.5uU/hr/100IEQ

- This increased insulin production persisted to 14 days post IGF-1(GPE) exposure. Longer duration's are yet to be investigated.

	14 days post IGF-1(GPE) Exposure	**3 days post IGF-1(GPE) Exposure**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline	1.3-fold increase Compared to control	1.5-fold increase Compared to control

- Withdrawal of Nicotinamide from the culture media eliminated the benefit of IGF-1(GPE) on islet insulin production.

	Incubated 3 days With 0.1ug/ml IGF-1(GPE) Without Nicotinamide	**Incubated 3 days With culture Media Without Nicotinamide**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 3 days culture Post isolation	47.6uU/hr/100IEQ	55.9uU/hr/100IEQ

- A concentration of 0.1ug/ml IGF-2 during culturing appeared to increase insulin production of porcine islet cells, after an initial exposure of 24 hrs. However, this increase was transient to 3 days post exposure.

	Incubated 24hrs With 0.1ug/ml IGF-2 day 1.	**Control**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline	105.8/100IEQ	75.2r/100IEQ

After 3 days culture
Post isolation

	Incubated 24hrs With 0.1ug/ml IGF-2 day 1.	**Control**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 7 days culture Post isolation	32uU/hr/100IEQ	39.8 uU/hr/100IEQ

- Prolonged exposure to IGF-2 beyond 24hrs, failed to increase the insulin production of the islet cells in response to glucose.

	Incubated 24hrs With 0.1ug/ml IGF-2 day 1.	**Control**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 3 days culture Post isolation	105.8/100IEQ	75.2r/100IEQ

	Incubated 7 days With 0.1ug/ml IGF-2	**Control**
Insulin secretion In response to 19.4mM Glucose + 10mM Theophyline After 7 days culture Post isolation	38.4uU/hr/100IEQ	39.8uU/hr/100IEQ

- **The effect of Lidocaine when used during porcine pancreatic digestion, on islet yield and viability.**

- Lidocaine is a membrane stabiliser and phospholipase A2 inhibitor. When used at a 1mM concentration during Collagenase digestion of 7d old porcine pancreas, a 2-fold increase in islet yield is produced.

- Islet endocrine function was assessed after 3 days in culture via static glucose stimulation. Islets isolated with Lidocaine during digestion produced a 3-fold increase in insulin secretion in response to glucose challenge.

	Collagenase alone	Collagenase + 1mM Lidocaine
Average islet yield	40,960 IEQ/g	88,183 IEQ/g

	Collagenase alone	Collagenase + 1mM Lidocaine
Insulin secretion in response to 19.4mM Glucose + 10mM Theophyline After 3 days culture Post isolation	46.4 uU/hr/100IEQ	163.8 uU/hr/100IEQ

Conclusion: The use of Lidocaine during pancreatic digestion increases the insulin production/g of pancreas by 6-fold.

Effects of Ciproxin on Islet function as assessed by static glucose stimulation.

Freshly prepared neonatal pig islets were prepared by standard isolation procedure and cultured for two days in RPMI medium with standard additions. Streptomycin (100mg/ml)and Penicillin (100U/ml) were included in one flask and Ciproxin (3 mcg/ml) in another.

The islets were harvested and an aliquot subjected to stimulation with theophylline and high glucose.

The comparative insulin release from the islets---a measure of viability is shown in the Figure below.

Effects of collagenase from various sources on islet yield and function

Pancreases of neonatal piglets aged 7 days were obtained as above and islets extracted by the same process, varying only the source and amount of collagenase. The yield/gram of pancreas is shown in the Figure.

Islets extracted using these variations in collagenase source and amount were assessed for viability using propidium iodide and dithizone for insulin content.

DTZ staining >85%

AO/PI >85%

The islets were then assessed for functionality by static glucose stimulation as above. The results are shown in the Figure below.

It is apparent that the Liberase™ preparations at suitable concentrations gave higher yields and function in vitro than the previously optimised concentration of

Collagenase P.

Comparative effectiveness of islets prepared with Liberase P or H in vivo

Islets prepared with the best concentration of Liberase P and H in this way were injected intraperitoneally into CD1 mice made diabetic by intravenous streptozotocin. The dose used was 10 islets/g body weight of mouse. Ten days after such treatment the number of mice no longer diabetic was assessed.

1/7 of the mice treated with the islets isolated with Liberase P and 4/7 of those islated with Liberase H were non diabetic.

Similar experiments were performed using spontaneously diabetic NOD mice. Of the surviving mice at 10 days after transplantation 3/7 of the Liberase P treated islets and 3/3 of the Liberase H islets were no longer diabetic

DATED THIS 15th DAY OF August 2000
AJ PARK
PER J. Feeney
AGENTS FOR THE APPLICANT

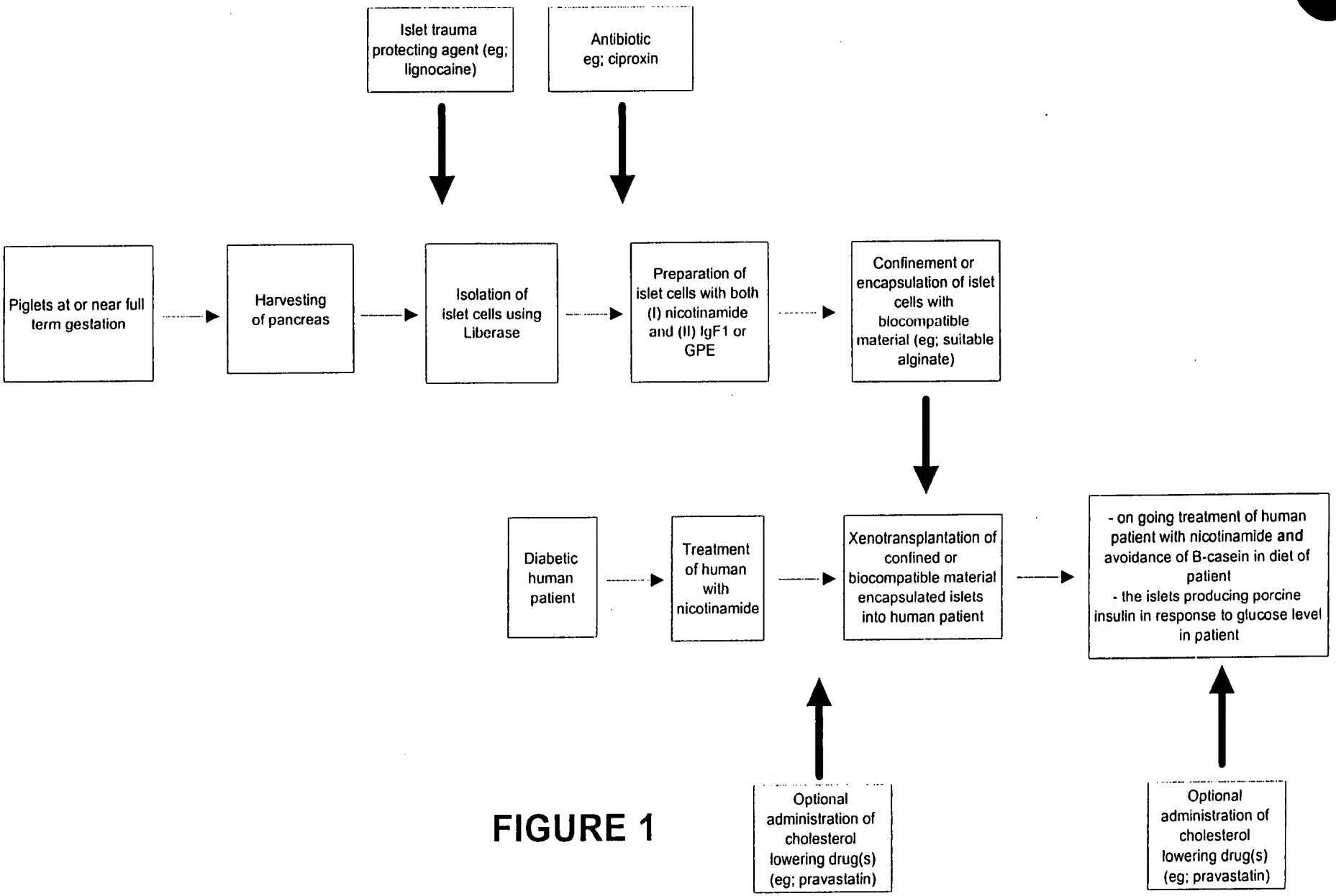

Islet trauma protecting agent (eg; lignocaine)
Antibiotic eg; ciproxin
Piglets at or near full term gestation
Harvesting of pancreas
Isolation of islet cells using Liberase
Preparation of islet cells with both (I) nicotinamide and (II) IgF1 or GPE
Confinement or encapsulation of islet cells with biocompatible material (eg; suitable alginate)
Diabetic human patient
Treatment of human with nicotinamide
Xenotransplantation of confined or biocompatible material encapsulated islets into human patient
- on going treatment of human patient with nicotinamide and avoidance of B-casein in diet of patient
- the islets producing porcine insulin in response to glucose level in patient
Optional administration of cholesterol lowering drug(s) (eg; pravastatin)
Optional administration of cholesterol lowering drug(s) (eg; pravastatin)

FIGURE 1

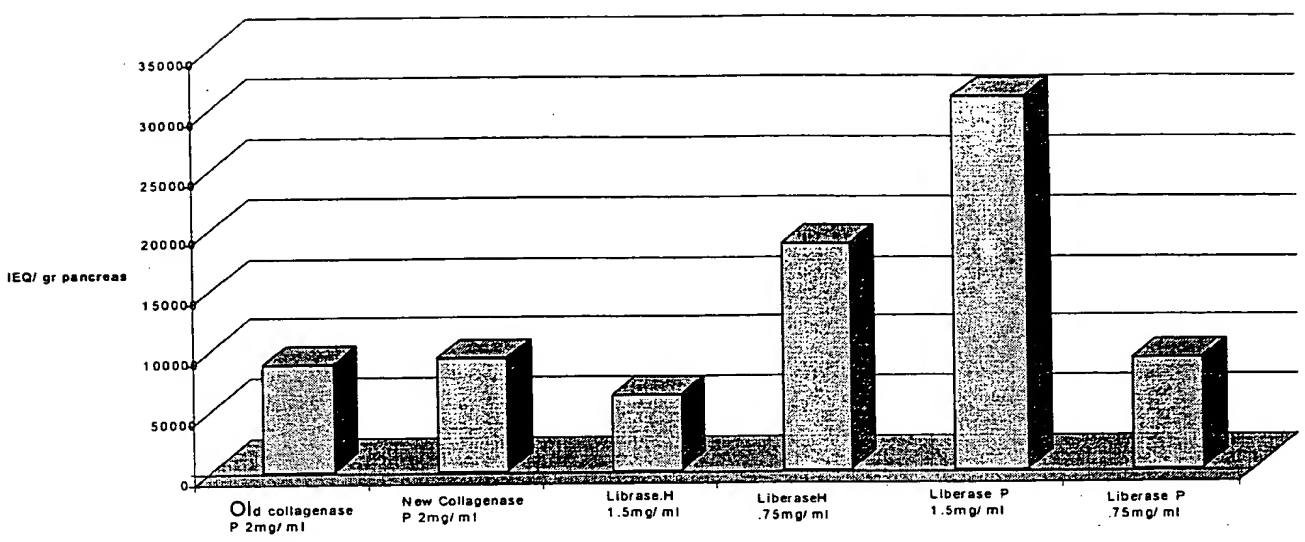


FIGURE 2

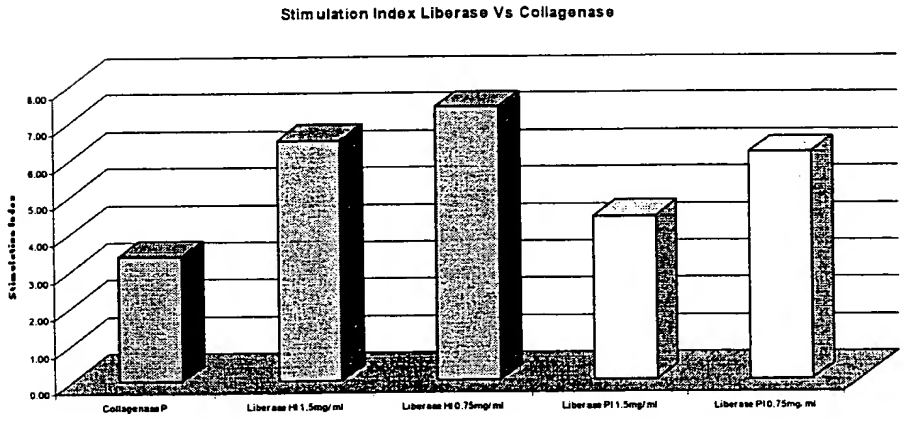


FIGURE 3

FIGURE 4

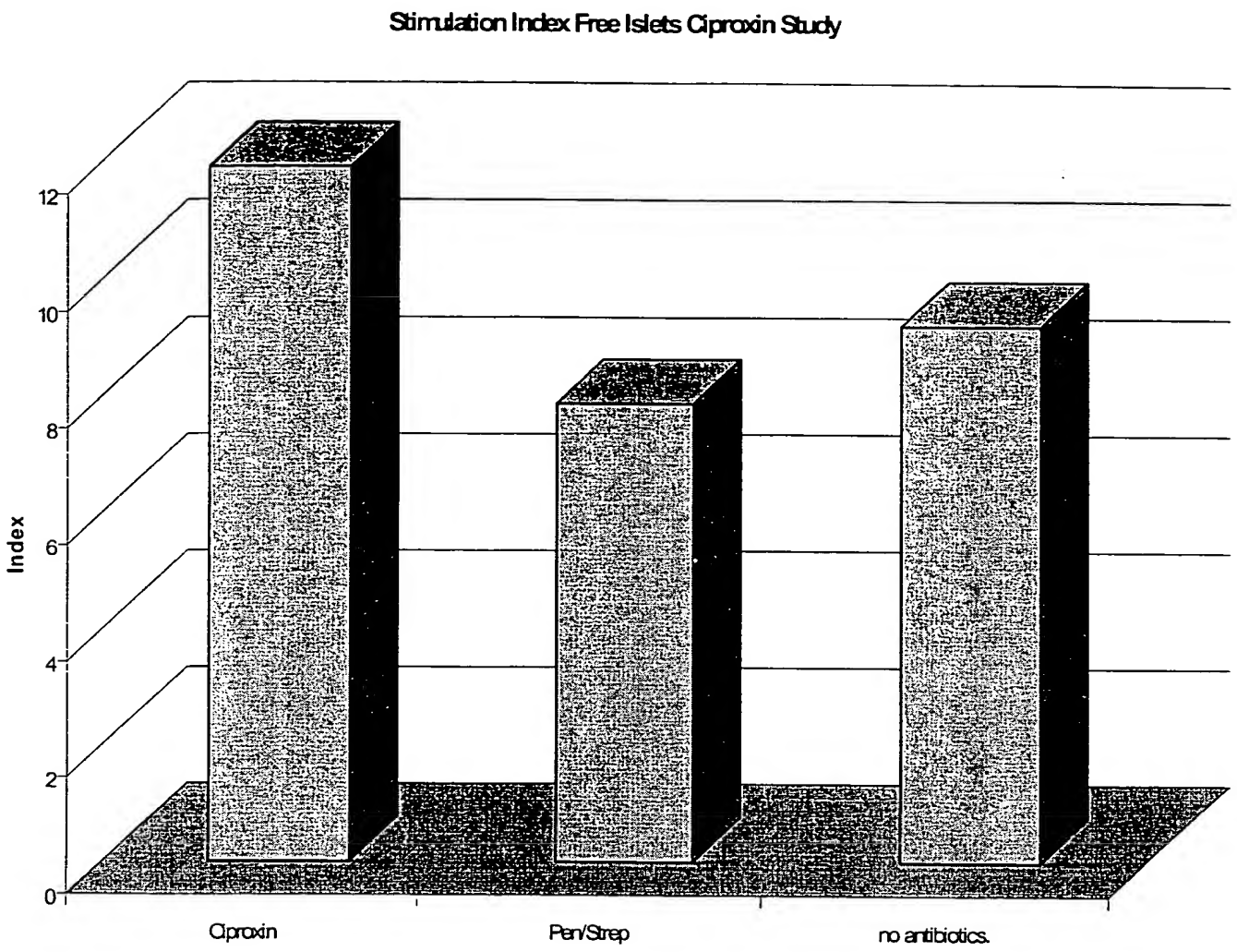
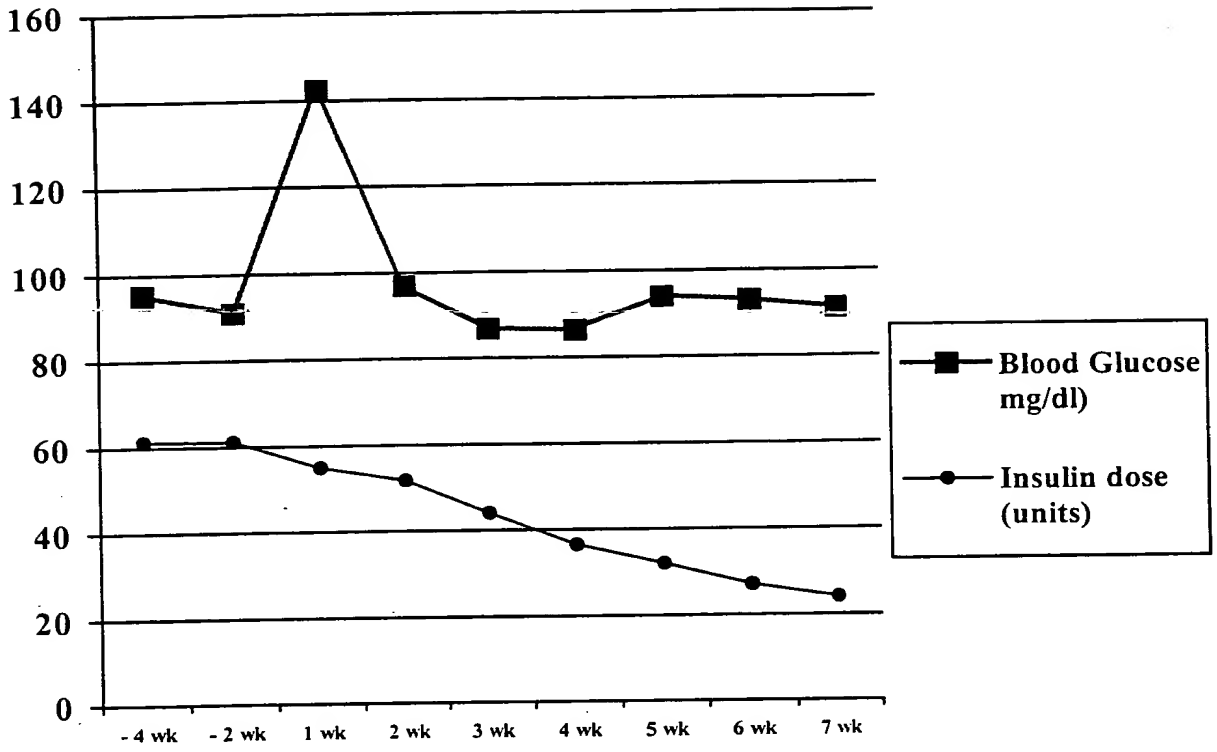

Stimulation Index Free Islets Ciproxin Study
Index
0
2
4
6
8
10
12
Ciproxin
Pen/Strep
no antibiotics.

FIGURE 5


160
140
120
100
80
60
40
20
0
- 4 wk
- 2 wk
1 wk
2 wk
3 wk
4 wk
5 wk
6 wk
7 wk
Blood Glucose mg/dl)
Insulin dose (units)

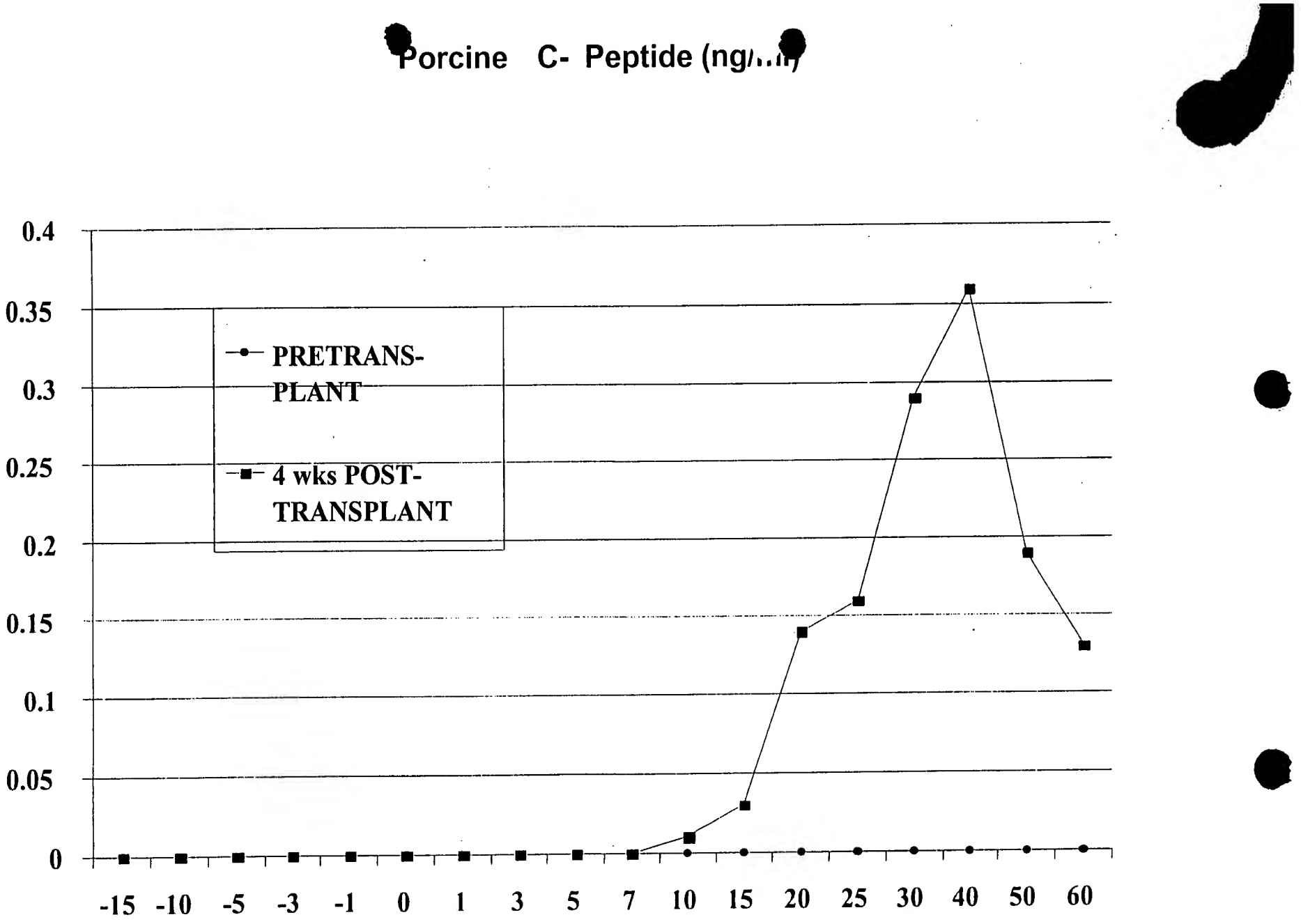

Porcine C- Peptide (ng/...)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PRETRANS-PLANT
4 wks POST-TRANSPLANT
-15 -10 -5 -3 -1 0 1 3 5 7 10 15 20 25 30 40 50 60

FIGURE 6